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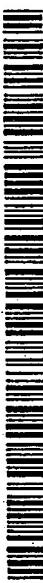
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(54) Title: LIPASE VARIANTS

(57) Abstract: Attaching a peptide extension to the C-terminal amino acid of a lipase reduces the tendency to form odor. This may lead to lipase variants with a reduced odor generation when washing textile soiled with fat which includes relatively short-chain fatty acyl groups (e.g. up to C8) such as dairy stains containing butter fat or tropical oils such as coconut oil or palm kernel oil.

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LIPASE VARIANTS

FIELD OF THE INVENTION

The present invention relates to lipase variants with reduced potential for odor generation and to a method of preparing them. It particularly relates to variants suited for use in 5 detergent compositions, more particularly variants of the *Thermomyces lanuginosus* lipase showing a first-wash effect and a reduced tendency to form odors when washing cloth soiled with milk fat.

BACKGROUND OF THE INVENTION

Lipases are useful, e.g., as detergent enzymes to remove lipid or fatty stains from 10 clothes and other textiles, as additives to dough for bread and other baked products. Thus, a lipase derived from *Thermomyces lanuginosus* (synonym *Humicola lanuginosa*, EP 258 068 and EP 305 216) is sold for detergent use under the tradename Lipolase® (product of Novo Nordisk A/S). WO 0060063 describes variants of the *T. lanuginosus* lipase with a particularly good first-wash performance in a detergent solution. WO 9704079, WO 9707202 and WO 15 0032758 also disclose variants of the *T. lanuginosus* lipase.

In some applications, it is of interest to minimize the formation of odor-generating short-chain fatty acids. Thus, it is known that laundry detergents with lipases may sometimes leave residual odors attached to cloth soiled with milk (EP 430315).

SUMMARY OF THE INVENTION

20 The inventors have found that attaching a peptide extension to the C-terminal amino acid of a lipase may reduce the tendency to form odor. This may lead to lipase variants with a reduced odor generation when washing textile soiled with fat which includes relatively short-chain fatty acyl groups (e.g. up to C₈) such as dairy stains containing butter fat or tropical oils such as coconut oil or palm kernel oil. The variants may have an increased specificity 25 for long-chain acyl groups over the short-chain acyl and/or an increased activity ratio at alkaline pH to neutral pH, i.e. a relatively low lipase activity at the neutral pH (around pH 7) during rinsing compared to the lipase activity at alkaline pH (e.g. pH 9 or 10) similar to the pH in a detergent solution.

Accordingly, the invention provides a method of producing a lipase by attaching a 30 peptide extension to the C-terminal of a parent lipase and screening resulting polypeptides for lipases with any of the above improved properties.

The invention also provides a polypeptide having lipase activity and having an amino acid sequence which comprises a parent polypeptide with lipase activity and a peptide extension attached to the C-terminal of the parent polypeptide.

UAMH (University of Alberta Mold Herbarium & Culture Collection), Devonian Botanic Garden, Edmonton, Alberta, Canada T6G 3G1.

Alternatively, the parent lipase may be a variant obtained by altering the amino acid sequence of any of the above lipases, particularly a variant having first-wash activity as described in WO 0060063 or as described below.

Peptide extension at C-terminal

The invention provides attachment of a peptide addition by a peptide bond to the C-terminal amino acid of a parent lipase (e.g. to L269 of the *T. lanuginosus* lipase shown as SEQ ID NO: 2). The peptide extension may be attached by site-directed or random mutagenesis.

The peptide extension at the C-terminal may consist of 2-15 amino acid residues, particularly 2-11 or 3-10, e.g. 2, 3, 4, 5, 7, 9 or 11 residues.

The extension may particularly have the following residues at the positions indicated (counting from the original C-terminal):

- 15 ▪ a negative amino acid residue (e.g. D or E) at the first position,
 ▪ a small, electrically uncharged amino acid (e.g. S, T, V or L) at the 2nd and/or
 the 3rd position, and/or
 ▪ a positive amino acid residue (e.g. H or K) at the 3rd-7th position , particularly
 the 4th, 5th or 6th.

20 The peptide extension may be HTPSSGRGGHR or a truncated form thereof, e.g.
HTPSSGRGG , HTPSSGR, HTPSS OR HTP. Other examples are KV, EST, LVY, RHT,
SVF, SVT, TAD, TPA, AGVF and PGLPKRVR.

25 The peptide extension may be attached by mutagenesis using a vector (a plasmid)
encoding the parent polypeptide and an oligonucleotide having a stop codon corresponding
to an extension of 2-15 amino acids from the C-terminal. The nucleotides between the C-
terminal and the stop codon may be random or may be biased to favor the amino acids de-
scribed above. One way of doing this would be to design a DNA oligo, which contains the
desired random mutations as well has the sequence necessary to hybridize to the 3^{end} of
the gene of interest. This DNA oligo is used in a PCR reaction along with an oligo with the
30 capability of hybridizing to the opposite DNA strand (as known to a person skilled in the art).
The PCR fragment is then cloned into the desired context (expression vector).

Increased long-chain/short-chain specificity

35 The lipase of the invention may have an increased long-chain/short-chain specificity
compared to the parent enzyme, e.g. an increased ratio of activity on long-chain (e.g. C₁₆-
C₂₀) triglycerides to the activity on short-chain (e.g. C₄-C₆) triglycerides. This may be deter-

Also, the lipase may have a negative or neutral net electric charge in the region 90-101 (particularly 94-101), i.e. the number of negative amino acids may be equal to or greater than the number of positive amino acids. Thus, the region may be unchanged from Lipolase, having two negative amino acids (D96 and E99) and one positive (K98), and having a neutral 5 amino acid at position 94 (N94), or the region may be modified by one or more substitutions.

Alternatively, two of the three amino acids N94, N96 and E99 may have a negative or unchanged electric charge. Thus, all three amino acids may be unchanged or may be changed by a conservative or negative substitution, i.e. N94(neutral or negative), D(negative) and E99(negative). Examples are N94D/E and D96E.

10 Further, one of the three amino acids N94, N96 and E99 may be substituted so as to increase the electric charge, i.e. N94(positive), D96(neutral or positive) or E99 (neutral or positive). Examples are N94K/R, D96I/L/N/S/W or E99N/Q/K/R/H.

The parent lipase may comprise a substitution corresponding to E99K combined with a negative amino acid in the region corresponding to 90-101, e.g. D96D/E.

15 The substitution of a neutral with a negative amino acid (N94D/E), may improve the performance in an anionic detergent. The substitution of a neutral amino acid with a positive amino acid (N94K/R) may provide a variant lipase with good performance both in an anionic detergent and in an anionic/non-ionic detergent (a detergent with e.g. 40-70 % anionic out of total surfactant).

20 Amino acids at other positions

The parent lipase may optionally comprise substitution of other amino acids, particularly less than 10 or less than 5 such substitutions. Examples are substitutions corresponding to Q249R/K/H, R209P/S and G91A in SEQ ID NO: 2. Further substitutions may, e.g., be made according to principles known in the art, e.g. substitutions described in WO 92/05249, 25 WO 94/25577, WO 95/22615, WO 97/04079 and WO 97/07202.

Parent lipase variants

The parent lipase may comprise substitutions corresponding to G91G/A +E99E/D/R/K +T231T/S/R/K +N233N/Q/R/K +Q249Q/N/R/K in SEQ ID NO: 2. Some particular examples are variants with substitutions corresponding to the following.

30

T231R+ N233R
D96L+ T231R+ N233R
G91A+ E99K+ T231R+ N233R+ Q249R
R209P +T231R +N233R
E87K +G91D +D96L +G225P +T231R +N233R +Q249R +N251D
G91A +E99K +T189G +T231R +N233R +Q249R

nal of Molecular Biology, 48, 443-45), using GAP with the following settings for polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

Amino acid sequence alignment

- In this specification, amino acid residues are identified by reference to SEQ ID NO:
- 5 2. To find corresponding positions in another lipase sequence, the sequence is aligned to SEQ ID NO: 2 by using the GAP alignment. GAP is provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-45). The following settings are used for
10 10 polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

DNA sequence, Expression vector, Host cell, Production of lipase

- The invention provides a DNA sequence encoding the lipase of the invention, an expression vector harboring the DNA sequence, and a transformed host cell containing the
15 DNA sequence or the expression vector. These may be obtained by methods known in the art.

The invention also provides a method of producing the lipase by culturing the transformed host cell under conditions conducive for the production of the lipase and recovering the lipase from the resulting broth. The method may be practiced according to principles
20 known in the art.

Lipase activity

Lipase activity on tributyrin at neutral and alkaline pH (LU7 and LU9)

A substrate for lipase is prepared by emulsifying tributyrin (glycerin tributyrate) using gum Arabic as emulsifier. The hydrolysis of tributyrin at 30 °C at pH 7 or 9 is followed in a
25 pH-stat titration experiment. One unit of lipase activity (1 LU7 or 1 LU9) equals the amount of enzyme capable of releasing 1 µmol butyric acid/min at pH 7 or 9. LU7 is also referred to as LU.

The relative lipase activity at neutral and alkaline pH may be expressed as LU9/LU7. This ratio may be at least 2.0.

30 Lipase activity on triolein (SLU)

The lipase activity is measured at 30°C and pH 9 with a stabilized olive oil emulsion (Sigma catalog No. 800-1) as the substrate, in a 5 mM Tris buffer containing 40 mM NaCl and 5 mM calcium chloride. 2.5 ml of the substrate is mixed with 12.5 ml buffer, the pH is ad-

Phosphonate [1-hydroxyethane-1,2-diylbis(phosphonic acid)]	0.1
Sodium perborate monohydrate	11.2
Tetraacetyl ethylenediamine (TAED)	6.3
Copoly(acrylic acid/maleic acid)	4.3
SRP (soil release polymer)	1.2

Detergent additive

According to the invention, the lipase may typically be used as an additive in a detergent composition. This additive is conveniently formulated as a non-dusting granulate, a stabilized liquid, a slurry or a protected enzyme. The additive may be prepared by methods known in the art.

DETERGENT COMPOSITION

The detergent compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the pretreatment of stained fabrics, rinse added fabric softener compositions, and compositions for use in general household hard surface cleaning operations and dishwashing operations.

The detergent composition of the invention comprises the lipase of the invention and a surfactant. Additionally, it may optionally comprise a builder, another enzyme, a suds suppresser, a softening agent, a dye-transfer inhibiting agent and other components conventionally used in detergents such as soil-suspending agents, soil-releasing agents, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or encapsulated or non-encapsulated perfumes.

The detergent composition according to the invention can be in liquid, paste, gel, bar, tablet or granular forms. The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, e.g. in the range of 7-11, particularly 9-11. Granular compositions according to the present invention can also be in "compact form", i.e. they may have a relatively higher density than conventional granular detergents, i.e. from 550 to 950 g/l.

The lipase of the invention, or optionally another enzyme incorporated in the detergent composition, is normally incorporated in the detergent composition at a level from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level from 0.01% to 0.2% of enzyme protein by weight of the composition.

A PCR reaction was made using oligo19671 and 991222j1 (SEQ ID NO: 11 and 12) with pENI1576 as template in a total of 100 µl using PWO polymerase (Boehringer Mannheim). Oligo 991222J1 adds 3 extra amino acids on the C-terminal.

- The PCR fragment was purified on a Biorad column and cut BamHI/SacII.
5 The plasmid pENI1861 (described in PCT/DK01/00805) was cut BamHI / SacII.
The PCR fragment and the plasmid vector was purified from a 1 % gel.
Vector and PCR fragment was ligated O/N, and electro-transformed into the *E.coli* strain DH10B giving 123,000 independent *E.coli* transformants.
10 independent clones were sequenced and showed satisfactory diversity.
10 A DNA-prep was made from all the clones.

Aspergillus transformation and screening.

Approximately 5 µg DNA plasmid was transformed into Jai355 (as mentioned in WO 00/24883). After 20 minutes incubation with PEG, the protoplasts were washed twice with 1.2 M sorbitol, 10 mM Tris pH7.5 (to remove CaCl₂).

- 15 The protoplasts were mixed in an alginate-solution (1.5 % alginate, 1 % dextran, 1.2 M sorbitol, 10 mM Tris pH 7.5). Using a pump (Ole Dich 110ACR.80G38.CH5A), this alginate solution dripped into a CaCl₂ – solution (1.2 M sorbitol, 10 mM Tris pH 7.5., 0.2 M CaCl₂) from a height of 15 cm. This created alginate beads of app. 2.5 mm in diameter with app. one transformed protoplast in every second bead. Approximately 55,000 transformants were
20 generated.

After the beads had been made, they were transferred to 1.2 M sorbitol, 10 mM Tris pH7.5, 10 mM CaCl₂ and grown o/n at 30°C. The beads were washed twice with sterile water and afterwards transferred to 1°vogel (without a carbon source, which is already present in the alginate-beads (dextran)). The beads grew o/w at 30°C.

- 25 After o/w growth, the beads were spread on plates containing TIDE and olive oil (1 g/L agarose, 0.1 M Tris pH 9.0, 5 mM CaCl₂, 25 ml/L olive oil, 1.4 g/L TIDE, 0.004 % brilliant green). The plates were incubated o/n at 37°C.

384 positive beads were transferred to four 96 well microtiter plates containing 150 µl 1°vogel, 2 % maltose in each well.

- 30 The plates were grown for 3 days at 34°C.
Media was assayed for activity towards pnp-valerate and pnp-palmitate at pH7.5 (as described in WO 00/24883)). The 64 clones having the highest activity on the long-chained substrate (pnp-palmitate) as well as low activity on the short chained substrate (pnp-valerate) were isolated on small plates, from which they were inoculated into a 96 well microtiter plate
35 containing 200 µl 1°vogel, 2 % maltose in each well.

After growth for 3 days at 34°C the media was once again assayed for activity towards pnp-valerate and pnp-palmitate at pH7.5 , as well as activity towards pnp-palmitate at pH10.

The lipase variant was added to the wash liquor at a dosage of 0.25 or 1.0 mg enzyme protein per liter. A control was made without addition of lipase variant, and a reference experiment was made with a lipase variant having the same amino acid sequence without any peptide extension.

5 The swatches were washed a second washing without lipase.

The performance was evaluated as follows:

- Odor generation was evaluated by a sensory panel, keeping the washed butter swatches in closed vials until the evaluation.
- Wash performance was evaluated by measuring the remission of the lard swatches after the first or the second washing. All variants showed a significant performance in this one-cycle washing test.
- A benefit/risk ratio was calculated as the performance on lard swatches after the first or second washing divided by the odor on butter swatches. An improved benefit/risk ratio indicates that the lipase can be dosed at a higher level than the reference to give wash performance on level with the reference with reduced odor.

All variants tested showed lower odor generation and/or a higher benefit/risk ratio than the same lipase without a peptide extension at the C-terminal.

Example 3: First-wash performance, activity at alkaline/neutral pH, long-chain/short-
20 chain activity

The following lipase variants based on SEQ ID NO: 2 were evaluated:

G91A +E99K +T231R +N233R +Q249R +270HTPSSGRGGH

G91A +E99K +T231R +N233R +Q249R +270HTPSSGRGG

G91A +E99K +T231R +N233R +Q249R +270HTPSSGR

25 G91A +E99K +T231R +N233R +Q249R +270HTPSS

G91A +E99K +T231R +N233R +Q249R +270EST

The first-wash performance was evaluated as described above, and each lipase variant was found to give a remission increase (ΔR) above 3.0.

30 The lipase activity was determined as LU7, LU9 and SLU by the methods described above. Each lipase variant was found to have a LU9/LU7 ratio above 2.0 and a SLU/LU9 ratio above 2.0.

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3	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on: page line	
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3-2		13-19
3-3	Identification of Deposit	
3-3-1	Name of depositary institution	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
3-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
3-3-3	Date of deposit	08 February 2001 (08.02.2001)
3-3-4	Accession Number	DSMZ 14049
3-4	Additional Indications	NONE
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3-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE
4	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on: page line	
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4-3-1	Name of depositary institution	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
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CLAIMS

1. A method of producing a polypeptide having lipase activity comprising:
 - a) preparing at least one polypeptide having an amino acid sequence which comprises:
 - 5 i) a parent polypeptide having lipase activity and,
 - ii) a peptide extension attached to the C-terminal of the parent polypeptide,
 - b) selecting a polypeptide which has lipase activity and which compared to the parent polypeptide has:
 - 10 i) a lower ratio between activities towards short-chain versus long-chain fatty acyl esters,
 - ii) a lower ratio between lipase activities at neutral versus alkaline pH, and/or
 - iii) a lower tendency to form odor in textile swatches with fatty soiling
 - 15 c) producing the selected polypeptide.
2. The method of claim 1 wherein the parent polypeptide has an amino acid sequence which has at least 50 % identity with SEQ ID NO: 2.
3. The method of claim 1 or 2 wherein the peptide extension consists of 2-15 amino acid residues, particularly 3-10.
- 20 4. The method of any of claims 1-3 wherein the peptide extension comprises a positive amino acid residue at position 4, 5 or 6.
5. The method of any of claims 1-4 wherein the polypeptide is prepared by mutagenesis using a plasmid encoding the parent polypeptide and an oligonucleotide having a stop codon corresponding to an extension of 2-15 amino acids.
- 25 6. A polypeptide having lipase activity and having an amino acid sequence which comprises:
 - a) a parent polypeptide having lipase activity and
 - b) a peptide extension comprising a positive, negative or polar amino acid residue attached to the C-terminal of the parent polypeptide.

16. The polypeptide of any of claims 6-15 wherein the peptide extension is HTPSSGRGGHR or a truncated form thereof (particularly HTPSSGRGG, HTPSSGR, HTPSS or HTP), KV, EST, LVY, RHT, SVF, SVT, TAD, TPA, AGVF or PGLPKRV.
17. A detergent composition comprising a surfactant and the polypeptide of any of claims 6-
5 16.
18. A DNA sequence encoding the polypeptide of any of claims 6-16.
19. An expression vector harboring the DNA sequence of claim 18.
20. A transformed host cell containing the DNA sequence of claim 18 or the expression vector of claim 19.
- 10 21. A method of producing the polypeptide of any of claims 6-16 which method comprises culturing the transformed host cell of claim 7 under conditions conducive for the production of the polypeptide and recovering the polypeptide from the resulting broth.
22. A detergent composition comprising a surfactant and a lipase which has:
15 a) a remission increase (ΔR) of at least 3 at the test washing conditions given in the specification,
b) a ratio of hydrolytic activities towards tributyrin at pH 9 and pH 7 (LU9/LU7) of at least 2.0, and
c) a ratio of hydrolytic activities towards olive oil and tributyrin (SLU/LU) of at least 2.0.
- 20 23. A method of preparing a detergent, comprising:
a) testing at least one lipase for:
i) its first-wash performance in a detergent solution,
ii) its relative lipase activity at neutral and alkaline pH, and
iii) its relative activity towards long-chain and short-chain acyl bonds in
25 triglycerides,
b) selecting a lipase which has:
i) a remission increase (ΔR) of at least 3 at the test washing conditions given in the specification,

SEQUENCE LISTING

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aca tgt ctt tagtggccgg cgccggctggg tccgactcta gcgagctcgat gatct 918
Thr Cys Leu

<210> 2

<211> 291

<212> PRT

<213> Thermomyces lanuginosus

<400> 2

Met Arg Ser Ser Leu Val Leu Phe Phe Val Ser Ala Trp Thr Ala Leu
-20 -15 -10

Ala Ser Pro Ile Arg Arg Glu Val Ser Gln Asp Leu Phe Asn Gln Phe
-5 -1 1 5 10

Asn Leu Phe Ala Glu Tyr Ser Ala Ala Ala Tyr Cys Gly Lys Asn Asn
15 20 25

Asp Ala Pro Ala Gly Thr Asn Ile Thr Cys Thr Gly Asn Ala Cys Pro
30 35 40

Glu Val Glu Lys Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser
45 50 55

Gly Val Gly Asp Val Thr Gly Phe Leu Ala Leu Asp Asn Thr Asn Lys
60 65 70

Leu Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Ile Glu Asn Trp Ile
75 80 85 90

Gly Asn Leu Asn Phe Asp Leu Lys Glu Ile Asn Asp Ile Cys Ser Gly
95 100 105

Cys Arg Gly His Asp Gly Phe Thr Ser Ser Trp Arg Ser Val Ala Asp
110 115 120

Thr Leu Arg Gln Lys Val Glu Asp Ala Val Arg Glu His Pro Asp Tyr
125 130 135

Arg Val Val Phe Thr Gly His Ser Leu Gly Gly Ala Leu Ala Thr Val
140 145 150

Ala Gly Ala-Asp Leu Arg Gly Asn Gly Tyr Asp Ile Asp Val Phe Ser
155 160 165 170

Val Gln Thr Gly Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile
190 195 200

Val Pro Arg Leu Pro Pro Arg Glu Phe Gly Tyr Ser His Ser Ser Pro
205 210 215

Glu Tyr Trp Ile Lys Ser Gly Thr Leu Val Pro Val Thr Arg Asn Asp
220 225 230

Ile Val Lys Ile Glu Gly Ile Asp Ala Thr Gly Gly Asn Asn Gln Pro
235 240 245 250

Asn Ile Pro Asp Ile Pro Ala His Leu Trp Tyr Phe Gly Leu Ile Gly
255 260 265

Thr Cys Leu

<210> 3

<211> 1083

<212> DNA

<213> Talaromyces thermophilus

<220>

<221> CDS

<222> (1)..(67)

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<222> (139)..(307)

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<222> (370)..(703)

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<222> (778)..(1080)

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<222> (67)..()

<223>

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gcc agt cct gtc cga cga g gtatgtaaat cacgggttat acttttcatg Ala Ser Pro Val Arg Arg	97
-5 -1	
cattgcatgt cgaacctgct gtactaagat tgcgcgacaca g ag gtc tcg cag gat Glu Val Ser Gln Asp	152
5	
ctg ttt gac cag ttc aac ctc ttt gcg cag tac tcg gcg gcc gca tac Leu Phe Asp Gln Phe Asn Leu Phe Ala Gln Tyr Ser Ala Ala Ala Tyr	200
10 15 20	
tgc gcg aag aac aac gat gcc ccg gca ggt ggg aac gta acg tgc agg Cys Ala Lys Asn Asn Asp Ala Pro Ala Gly Gly Asn Val Thr Cys Arg	248
25 30 35	
gga agt att tgc ccc gag gta gag aag gcg gat gca acg ttt ctc tac Gly Ser Ile Cys Pro Glu Val Glu Lys Ala Asp Ala Thr Phe Leu Tyr	296
40 45 50	
tcg ttt gag ga gtaggtgtca acaagagtac aggcacccgt agtagaaata Ser Phe Glu Asp	347
55	
gcagactaac tggaaatgt ag t tct gga gtt ggc gat gtc acc ggg ttc Ser Gly Val Gly Asp Val Thr Gly Phe	397
60 65	
ctt gct ctc gac aac acg aac aga ctg atc gtc ctc tct ttc cgc ggc Leu Ala Leu Asp Asn Thr Asn Arg Leu Ile Val Leu Ser Phe Arg Gly	445
70 75 80	
tct cgt tcc ctg gaa aac tgg atc ggg aat atc aac ttg gac ttg aaa Ser Arg Ser Leu Glu Asn Trp Ile Gly Asn Ile Asn Leu Asp Leu Lys	493
85 90 95	
gga att gac gac atc tgc tct ggc tgc aag gga cat gac ggc ttc act Gly Ile Asp Asp Ile Cys Ser Gly Cys Lys Gly His Asp Gly Phe Thr	541
100 105 110	
tcc tcc tgg agg tcc gtt gcc aat acc ttg act cag caa gtg cag aat Ser Ser Trp Arg Ser Val Ala Asn Thr Leu Thr Gln Gln Val Gln Asn	589
115 120 125 130	
gct gtg agg gag cat ccc gac tac cgc gtc gtc ttc act ggg cac agc	637

Ala Val Arg Glu His Pro Asp Tyr Arg Val Val Phe Thr Gly His Ser 135 140 145	
ttg ggt ggt gca ttg gca act gtg gcc ggg gca tct ctg cgt gga aat Leu Gly Gly Ala Leu Ala Thr Val Ala Gly Ala Ser Leu Arg Gly Asn 150 155 160	685
ggg tac gat ata gat gtg gtatgttagga aaaatgatcc ccgtggagcg Gly Tyr Asp Ile Asp Val 165	733
gtcatgtgga aatgtgcagg ggtgtcta at acacagacca acag ttc tca tat ggc Phe Ser Tyr Gly 170	789
gct ccc cgc gtc gga aac agg gct ttt gcg gaa ttc ctg acc gca cag Ala Pro Arg Val Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr Ala Gln 175 180 185	837
acc ggc ggc acc ttg tac cgc atc acc cac acc aat gat att gtc ccc Thr Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile Val Pro 190 195 200	885
aga ctc ccg cca cgc gaa ttg ggt tac agc cat tct agc cca gag tat Arg Leu Pro Pro Arg Glu Leu Gly Tyr Ser His Ser Ser Pro Glu Tyr 205 210 215 220	933
tgg atc acg tct gga acc ctc gtc cca gtg acc aag aac gat atc gtc Trp Ile Thr Ser Gly Thr Leu Val Pro Val Thr Lys Asn Asp Ile Val 225 230 235	981
aag gtg gag ggc atc gat tcc acc gat gga aac aac cag cca aat acc Lys Val Glu Gly Ile Asp Ser Thr Asp Gly Asn Asn Gln Pro Asn Thr 240 245 250	1029
ccg gac att gct gcg cac cta tgg tac ttc ggg tca atg gcg acg tgt Pro Asp Ile Ala Ala His Leu Trp Tyr Phe Gly Ser Met Ala Thr Cys 255 260 265	1077
ttg taa Leu	1083

<210> 4

<211> 291

<212> PRT

<213> Talaromyces thermophilus

<400> 4

Met Arg Ser Ser Leu Val Leu Phe Phe Val Ser Ala Trp Thr Ala Leu
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-5 -1 1 5 10Asn Leu Phe Ala Gln Tyr Ser Ala Ala Ala Tyr Cys Ala Lys Asn Asn
15 20 25

Asp Ala Pro Ala Gly Gly Asn Val Thr Cys Arg Gly Ser Ile Cys Pro
30 35 40

Glu Val Glu Lys Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser
45 50 55

Gly Val Gly Asp Val Thr Gly Phe Leu Ala Leu Asp Asn Thr Asn Arg
60 65 70

Leu Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Leu Glu Asn Trp Ile
75 80 85 90

Gly Asn Ile Asn Leu Asp Leu Lys Gly Ile Asp Asp Ile Cys Ser Gly
95 100 105

Cys Lys Gly His Asp Gly Phe Thr Ser Ser Trp Arg Ser Val Ala Asn
110 115 120

Thr Leu Thr Gln Gln Val Gln Asn Ala Val Arg Glu His Pro Asp Tyr
125 130 135

Arg Val Val Phe Thr Gly His Ser Leu Gly Gly Ala Leu Ala Thr Val
140 145 150

Ala Gly Ala Ser Leu Arg Gly Asn Gly Tyr Asp Ile Asp Val Phe Ser
155 160 165 170

Tyr Gly Ala Pro Arg Val Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr
175 180 185

Ala Gln Thr Gly Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile
190 195 200

Val Pro Arg Leu Pro Pro Arg Glu Leu Gly Tyr Ser His Ser Ser Pro
205 210 215

Glu Tyr Trp Ile Thr Ser Gly Thr Leu Val Pro Val Thr Lys Asn Asp
220 225 230

Ile Val Lys Val Glu Gly Ile Asp Ser Thr Asp Gly Asn Asn Gln Pro
235 240 245 250

Asn Thr Pro Asp Ile Ala Ala His Leu Trp Tyr Phe Gly Ser Met Ala
255 260 265

Thr Cys Leu

<210> 5

<211> 1070

<212> DNA

<213> Thermomyces ibadanensis

<220>

<221> CDS

<222> (1)..(67)

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<221> CDS

<222> (128)..(296)

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<222> (765)..(1067)

<223>

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<221> mat_peptide

<222> (67)..()

<223>

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Met Arg Ser Ser Leu Val Leu Phe Phe Leu Ser Ala Trp Thr Ala Leu
-20 -15 -10

gcg cgg cct gtt cga cga g gtatgttagca agggacacta ttacatgttg 97
Ala Arg Pro Val Arg Arg
-5 -1

accttggta ttctaaact gcatgcgcag cg gtt ccg caa gat ctg ctc gac 150
Ala Val Pro Gln Asp Leu Leu Asp
5

cag ttt gaa ctc ttt tca caa tat tcg gcg gcc gca tac tgt gcg gca Gln Phe Glu Leu Phe Ser Gln Tyr Ser Ala Ala Ala Tyr Cys Ala Ala 10 15 20	198
aac aat cat gct cca gtc ggc tca gac gta acg tgc tcg gag aat gtc Asn Asn His Ala Pro Val Gly Ser Asp Val Thr Cys Ser Glu Asn Val 25 30 35 40	246
tgc cct gag gta gat gcg gcg gac gca acg ttt ctc tat tct ttt gaa Cys Pro Glu Val Asp Ala Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu 45 50 55	294
ga gtgggtgtcg acaaaggcaca gagacagttag tagagacagc agtctaactg Asp	346
agatgtgcag t tct gga tta ggc gat gtt acc ggc ctt ctc gct ctc gac Ser Gly Leu Gly Asp Val Thr Gly Leu Leu Ala Leu Asp 60 65 70	396
aac acg aat aaa ctg atc gtc ctc tct ttc cgc ggc tct cgc tca gta Asn Thr Asn Lys Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Val 75 80 85	444
gag aac tgg atc gcg aac ctc gcc gcc gac ctg aca gaa ata tct gac Glu Asn Trp Ile Ala Asn Leu Ala Asp Leu Thr Glu Ile Ser Asp 90 95 100	492
atc tgc tcc ggc tgc gag ggg cat gtc ggc ttc gtt act tct tgg agg Ile Cys Ser Gly Cys Glu Gly His Val Gly Phe Val Thr Ser Trp Arg 105 110 115	540
tct gta gcc gac act ata agg gag cag gtg cag aat gcc gtg aac gag Ser Val Ala Asp Thr Ile Arg Glu Gln Val Gln Asn Ala Val Asn Glu 120 125 130	588
cat ccc gat tac cgc gtg gtc ttt acc gga cat agc ttg gga ggc gca His Pro Asp Tyr Arg Val Val Phe Thr Gly His Ser Leu Gly Ala 135 140 145 150	636
ctg gca act att gcc gca gca gct ctg cga gga aat gga tac aat atc Leu Ala Thr Ile Ala Ala Ala Leu Arg Gly Asn Gly Tyr Asn Ile 155 160 165	684
gac gtg gtatgtggga agaaggccacc cagacaaaca attatgtgga aacatgcaag Asp Val	740
gatggctaat acacggtcca acag ttc tca tat ggc gcg ccc cgc gtc ggt Phe Ser Tyr Gly Ala Pro Arg Val Gly 170 175	791
aac agg gca ttt gca gaa ttc ctg acc gca cag acg ggc ggc acc ctg Asn Arg Ala Phe Ala Glu Phe Leu Thr Ala Gln Thr Gly Gly Thr Leu 180 185 190	839
tat cgc atc acc cat acc aat gat atc gtc cct aga ctc cct cct cga Tyr Arg Ile Thr His Thr Asn Asp Ile Val Pro Arg Leu Pro Pro Arg 195 200 205	887
gac tgg got tac agc cac tct agc ccg gag tac tgg gtc acg tct ggt Asp Trp Gly Tyr Ser His Ser Ser Pro Glu Tyr Trp Val Thr Ser Gly 210 215 220 225	935
aac gac gtc cca gtg acc gca aac gac atc acc gtc gtg gag ggc atc Asn Asp Val Pro Val Thr Ala Asn Asp Ile Thr Val Val Glu Gly Ile 230 235 240	983

gat tcc acc gac ggg aac aac cag ggg aat atc cca gac atc cct tcg 1031
 Asp Ser Thr Asp Gly Asn Asn Gln Gly Asn Ile Pro Asp Ile Pro Ser
 245 250 255

cat cta tgg tat ttc got ccc att tca gag tgt gat tag 1070
 His Leu Trp Tyr Phe Gly Pro Ile Ser Glu Cys Asp
 260 265

<210> 6

<211> 291

<212> PRT

<213> Thermomyces ibadanensis

<400> 6

Met Arg Ser Ser Leu Val Leu Phe Phe Leu Ser Ala Trp Thr Ala Leu
 -20 -15 -10

Ala Arg Pro Val Arg Arg Ala Val Pro Gln Asp Leu Leu Asp Gln Phe
 -5 -1 1 5 10

Glu Leu Phe Ser Gln Tyr Ser Ala Ala Ala Tyr Cys Ala Ala Asn Asn
 15 20 25

His Ala Pro Val Gly Ser Asp Val Thr Cys Ser Glu Asn Val Cys Pro
 30 35 40

Glu Val Asp Ala Ala Asp Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser
 45 50 55

Gly Leu Gly Asp Val Thr Gly Leu Leu Ala Leu Asp Asn Thr Asn Lys
 60 65 70

Leu Ile Val Leu Ser Phe Arg Gly Ser Arg Ser Val Glu Asn Trp Ile
 75 80 85 90

Ala Asn Leu Ala Ala Asp Leu Thr Glu Ile Ser Asp Ile Cys Ser Gly
 95 100 105

Cys Glu Gly His Val Gly Phe Val Thr Ser Trp Arg Ser Val Ala Asp
 110 115 120

Thr Ile Arg Glu Gln Val Gln Asn Ala Val Asn Glu His Pro Asp Tyr
 125 130 135

Arg Val Val Phe Thr Gly His Ser Leu Gly Gly Ala Leu Ala Thr Ile
 140 145 150

Ala Ala Ala Ala Leu Arg Gly Asn Gly Tyr Asn Ile Asp Val Phe Ser
 155 160 165 170

Tyr Gly Ala Pro Arg Val Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr
175 180 185

Ala Gln Thr Gly Gly Thr Leu Tyr Arg Ile Thr His Thr Asn Asp Ile
190 195 200

Val Pro Arg Leu Pro Pro Arg Asp Trp Gly Tyr Ser His Ser Ser Pro
205 210 215

Glu Tyr Trp Val Thr Ser Gly Asn Asp Val Pro Val Thr Ala Asn Asp
220 225 230

Ile Thr Val Val Glu Gly Ile Asp Ser Thr Asp Gly Asn Asn Gln Gly
235 240 245 250

Asn Ile Pro Asp Ile Pro Ser His Leu Trp Tyr Phe Gly Pro Ile Ser
255 260 265

Glu Cys Asp

<210> 7

<211> 1064

<212> DNA

<213> Talaromyces emersonii

<220>

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<222> (1)..(88)

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<220>

<221> mat_peptide

<222> (88)..()

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<221> CDS

<222> (142)..(310)

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<221> CDS

<222> (362)..(695)

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<221> CDS

<222> (756)..(1061)

<223>

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 Met Phe Lys Ser Ala Ala Val Arg Ala Ile Ala Ala Leu Gly Leu Thr
 -25 -20 -15

gcg tca gtc ttg gct gct cct gtt gaa ctg ggc cgt cga g gtaaggaagc 98
 Ala Ser Val Leu Ala Ala Pro Val Glu Leu Gly Arg Arg
 -10 -5 -1

atgacggaga gaacaccctg tgcgacctgc tgacatccctt cag at gtt tct cag 152
 Asp Val Ser Gln

gac ctc ttc gac cag ctc aat ctt ttc gag cag tac tcg gcg gct gcg 200
 Asp Leu Phe Asp Gln Leu Asn Leu Phe Glu Gln Tyr Ser Ala Ala Ala
 5 10 15 20

tac tgt tca gct aac aat gag gcc tct gcc ggc acg gca atc tct tgc 248
 Tyr Cys Ser Ala Asn Asn Glu Ala Ser Ala Gly Thr Ala Ile Ser Cys
 25 30 35

tcc gca ggc aat tgc ccg ttg gtc cag cag gct gga gca acc atc ctg 296
 Ser Ala Gly Asn Cys Pro Leu Val Gln Gln Ala Gly Ala Thr Ile Leu
 40 45 50

tat tca ttc aac aa gtgggtgtca cgaaaaagat tggataacc aacatgttga 350
 Tyr Ser Phe Asn Asn
 55

cgttgtgtca g c att ggc tct ggc gat gtg acg ggt ttt ctc gct ctc 398
 Ile Gly Ser Gly Asp Val Thr Gly Phe Leu Ala Leu
 60 65

gac tcg acg aat caa ttg atc gtc ttg tca ttc cgg gga tca gag act 446
 Asp Ser Thr Asn Gln Leu Ile Val Leu Ser Phe Arg Gly Ser Glu Thr
 70 75 80 85

ctc gaa aac tgg atc gct gac ctg gaa gct gac ctg gtc gat gcc tct 494
 Leu Glu Asn Trp Ile Ala Asp Leu Glu Ala Asp Leu Val Asp Ala Ser
 90 95 100

gcc atc tgt tcc ggc tgt gaa gca cac gat ggg ttc ctt tca tcc tgg 542
 Ala Ile Cys Ser Gly Cys Glu Ala His Asp Gly Phe Leu Ser Ser Trp
 105 110 115

aat tca gtc gcc agc act ctg aca tcc aaa atc tcg tcg gcc gtc aac 590

Asn Ser Val Ala Ser Thr Leu Thr Ser Lys Ile Ser Ser Ala Val Asn.			
120	125	130	
gaa cat ccc agc tac aag ctg gtc ttc acc ggc cac agt ctc gga gcc Glu His Pro Ser Tyr Lys Leu Val Phe Thr Gly His Ser Leu Gly Ala		135	638
140	145		
gcc ttg gct aca ctt gga gcc gtt tct ctt aga gag agc gga tat aat Ala Leu Ala Thr Leu Gly Ala Val Ser Leu Arg Glu Ser Gly Tyr Asn		150	686
155	160	165	
att gac ctc gtaagttcc ggcacggcg tcgtcatcat cgagcggaaa Ile Asp Leu			735
gactgaccgg ttaactgcag tac aat tat ggc tgc ccc cgg gtc ggt aac acc Tyr Asn Tyr Gly Cys Pro Arg Val Gly Asn Thr		170	788
175			
gcg ctc gca gac ttc atc acc acg caa tcc gga ggc aca aat tac cgc Ala Leu Ala Asp Phe Ile Thr Thr Gln Ser Gly Gly Thr Asn Tyr Arg		180	836
185	190	195	
gtc acg cat tcc gat gac cct gtc ccc aag ctg cct ccc agg agt ttt Val Thr His Ser Asp Asp Pro Val Pro Lys Leu Pro Pro Arg Ser Phe		200	884
205	210		
gga tac agc caa ccg agc cca gag tac tgg atc acc tca ggg aac aat Gly Tyr Ser Gln Pro Ser Pro Glu Tyr Trp Ile Thr Ser Gly Asn Asn		215	932
220	225		
gta act gtt caa ccg tcc gac atc gag gtc atc gaa ggc gtc gac tcc Val Thr Val Gln Pro Ser Asp Ile Glu Val Ile Glu Gly Val Asp Ser		230	980
235	240		
act gca ggc aac gac ggc acc cct gct ggc ctt gac att gat gct cat Thr Ala Gly Asn Asp Gly Thr Pro Ala Gly Leu Asp Ile Asp Ala His		245	1028
250	255		
cgg tgg tac ttt gga ccc att agc gca tgt tcg tga Arg Trp Tyr Phe Gly Pro Ile Ser Ala Cys Ser		260	1064
265	270		
<210> 8			
<211> 299			
<212> PRT			
<213> Talaromyces emersonii			
<400> 8			
Met Phe Lys Ser Ala Ala Val Arg Ala Ile Ala Ala Leu Gly Leu Thr			
-25	-20	-15	
Ala Ser Val Leu Ala Ala Pro Val Glu Leu Gly Arg Arg Asp Val Ser			
-10	-5	-1 1	
Gln Asp Leu Phe Asp Gln Leu Asn Leu Phe Glu Gln Tyr Ser Ala Ala			
5	10	15	

Ala Tyr Cys Ser Ala Asn Asn Glu Ala Ser Ala Gly Thr Ala Ile Ser
20 25 30 35

Cys Ser Ala Gly Asn Cys Pro Leu Val Gln Gln Ala Gly Ala Thr Ile
40 45 50

Leu Tyr Ser Phe Asn Asn Ile Gly Ser Gly Asp Val Thr Gly Phe Leu
55 60 65

Ala Leu Asp Ser Thr Asn Gln Leu Ile Val Leu Ser Phe Arg Gly Ser
70 75 80

Glu Thr Leu Glu Asn Trp Ile Ala Asp Leu Glu Ala Asp Leu Val Asp
85 90 95

Ala Ser Ala Ile Cys Ser Gly Cys Glu Ala His Asp Gly Phe Leu Ser
100 105 110 115

Ser Trp Asn Ser Val Ala Ser Thr Leu Thr Ser Lys Ile Ser Ser Ala
120 125 130

Val Asn Glu His Pro Ser Tyr Lys Leu Val Phe Thr Gly His Ser Leu
135 140 145

Gly Ala Ala Leu Ala Thr Leu Gly Ala Val Ser Leu Arg Glu Ser Gly
150 155 160

Tyr Asn Ile Asp Leu Tyr Asn Tyr Gly Cys Pro Arg Val Gly Asn Thr
165 170 175

Ala Leu Ala Asp Phe Ile Thr Thr Gln Ser Gly Gly Thr Asn Tyr Arg
180 185 190 195

Val Thr His Ser Asp Asp Pro Val Pro Lys Leu Pro Pro Arg Ser Phe
200 205 210

Gly Tyr Ser Gln Pro Ser Pro Glu Tyr Trp Ile Thr Ser Gly Asn Asn
215 220 225

Val Thr Val Gln Pro Ser Asp Ile Glu Val Ile Glu Gly Val Asp Ser
230 235 240

Thr Ala Gly Asn Asp Gly Thr Pro Ala Gly Leu Asp Ile Asp Ala His
245 250 255

Arg Trp Tyr Phe Gly Pro Ile Ser Ala Cys Ser
260 265 270

<210> 9

<211> 1074

<212> DNA

<213> *Talaromyces byssochlamydooides*

<220>

<221> CDS

<222> (1)..(85)

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<222> (150)..(318)

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<221> CDS

<222> (760)..(1071)

<223>

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<221> mat_peptide

<222> (85)..()

<223>

<400> 9

atg ttc aaa tca act gtc cgg gcc atc gcc gca ctc gga ctg acc tcg	48
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-25	-20
	-15

tca gtc ttt gct gct cct atc gaa ctg ggc cgt cga g gtaagggca	95
Ser Val Phe Ala Ala Pro Ile Glu Leu Gly Arg Arg	
-10	-5
	-1

tgaaaactcc ctgtatggca tctcatctgg cagcatatct actgacatcc tcag at	151
Asp	

gtt tcg gag cag ctc ttc aac cag ttc aat ctc ttc gag cag tat tcc Val Ser Glu Gln Leu Phe Asn Gln Phe Asn Leu Phe Glu Gln Tyr Ser 5 10 15	199
gcg gct gct tac tgt cca gcc aac ttt gag tcc gct tcc ggc gca Ala Ala Ala Tyr Cys Pro Ala Asn Phe Glu Ser Ala Ser Gly Ala Ala 20 25 30	247
att tct tgt tcc aca ggc aat tgc ccc ctc gtc caa cag gct ggc gca Ile Ser Cys Ser Thr Gly Asn Cys Pro Leu Val Gln Gln Ala Gly Ala 35 40 45	295
acc acc ctg tat gca ttc aac aa gtgagtgtca tgaaaggct tgttggata Thr Thr Leu Tyr Ala Phe Asn Asn 50 55	348
ccgtacgggt atgttgactg tcatcag c atc ggc tct ggc gat gtg acg ggt Ile Gly Ser Gly Asp Val Thr Gly 60 65	400
ttt ctt gct gtc gat ccg acc aac cga ctc atc gtc ttg tcg ttc cgg Phe Leu Ala Val Asp Pro Thr Asn Arg Leu Ile Val Leu Ser Phe Arg 70 75 80	448
ggg tca gag agt ctc gag aac tgg atc act aat ctc agc gac gcc gac ctg Gly Ser Glu Ser Leu Glu Asn Trp Ile Thr Asn Leu Ser Ala Asp Leu 85 90 95	496
gtc gat gcc tct gca atc tgt tcc ggg tgt gaa gcc cat gac gga ttc Val Asp Ala Ser Ala Ile Cys Ser Gly Cys Glu Ala His Asp Gly Phe 100 105 110	544
tat tcg tct tggcaa tca gtt gcc agc act ctg acc tcc caa atc tcg Tyr Ser Ser Trp Gln Ser Val Ala Ser Thr Leu Thr Ser Gln Ile Ser 115 120 125	592
tcg gcc ctc tcg gca tat cca aac tac aag ctg gtc ttc acc ggc cac Ser Ala Leu Ser Ala Tyr Pro Asn Tyr Lys Leu Val Phe Thr Gly His 130 135 140 145	640
agt ctc gga gcc gca tta gct aca ctt gga gct gtc tct ctc agg gag Ser Leu Gly Ala Ala Leu Ala Thr Leu Gly Ala Val Ser Leu Arg Glu 150 155 160	688
agt gga tac aat atc gac ctc gtaagttcct ggcattgcca tcatggaaag Ser Gly Tyr Asn Ile Asp Leu 165	739
agactcacag ttaactgttag tac aac tti ggc tgt ccc cggt gtc ggc aac act Tyr Asn Phe Gly Cys Pro Arg Val Gly Asn Thr 170 175	792
gct ctc gca gac ttt att acc aac caa acc ggt ggc aca aat tac cgg Ala Leu Ala Asp Phe Ile Thr Asn Gln Thr Gly Gly Thr Asn Tyr Arg 180 185 190 195	840
gta acg cat tac gag gac cct gtc ccc aag ctg cct ccc agg agt ttt Val Thr His Tyr Glu Asp Pro Val Pro Lys Leu Pro Pro Arg Ser Phe 200 205 210	888
gga tac agc caa cct agc ccg gaa tac tgg atc acg tcg gga aac aat Gly Tyr Ser Gln Pro Ser Pro Glu Tyr Trp Ile Thr Ser Gly Asn Asn 215 220 225	936
gtg act gtg act tcg tcc gac atc gat gtc gtc gtg ggt gtc gac tcg Val Thr Val Thr Ser Ser Asp Ile Asp Val Val Val Gly Val Asp Ser 230 235 240	984

act gca ggc aac gac ggg acg cct gat ggc ctt gac act gct gcc cat 1032
 Thr Ala Gly Asn Asp Gly Thr Pro Asp Gly Leu Asp Thr Ala Ala His
 245 250 255
 agg tgg tat ttt gga cct act acc gaa tgt tcg tcg tca tga 1074
 Arg Trp Tyr Phe Gly Pro Thr Thr Glu Cys Ser Ser Ser
 260 265 270

<210> 10
 <211> 300
 <212> PRT
 <213> Talaromyces byssochlamydooides

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 -25

Ser Val Phe Ala Ala Pro Ile Glu Leu Gly Arg Arg Asp Val Ser Glu -1
 -10 -5 -1 1

Gln Leu Phe Asn Gln Phe Asn Leu Phe Glu Gln Tyr Ser Ala Ala Ala
 5 10 15 20

Tyr Cys Pro Ala Asn Phe Glu Ser Ala Ser Gly Ala Ala Ile Ser Cys
 25 30 35

Ser Thr Gly Asn Cys Pro Leu Val Gln Gln Ala Gly Ala Thr Thr Leu
 40 45 50

Tyr Ala Phe Asn Asn Ile Gly Ser Gly Asp Val Thr Gly Phe Leu Ala
 55 60 65

Val Asp Pro Thr Asn Arg Leu Ile Val Leu Ser Phe Arg Gly Ser Glu
 70 75 80

Ser Leu Glu Asn Trp Ile Thr Asn Leu Ser Ala Asp Leu Val Asp Ala
 85 90 95 100

Ser Ala Ile Cys Ser Gly Cys Glu Ala His Asp Gly Phe Tyr Ser Ser
 105 110 115

Trp Gln Ser Val Ala Ser Thr Leu Thr Ser Gln Ile Ser Ser Ala Leu
 120 125 130

Ser Ala Tyr Pro Asn Tyr Lys Leu Val Phe Thr Gly His Ser Leu Gly
 135 140 145

Ala Ala Leu Ala Thr Leu Gly Ala Val Ser Leu Arg Glu Ser Gly Tyr
 150 155 160

Asn Ile Asp Leu Tyr Asn Phe Gly Cys Pro Arg Val Gly Asn Thr Ala
165 170 175 180

Leu Ala Asp Phe Ile Thr Asn Gln Thr Gly Gly Thr Asn Tyr Arg Val
185 190 195

Thr His Tyr Glu Asp Pro Val Pro Lys Leu Pro Pro Arg Ser Phe Gly
200 205 210

Tyr Ser Gln Pro Ser Pro Glu Tyr Trp Ile Thr Ser Gly Asn Asn Val
215 220 225

Thr Val Thr Ser Ser Asp Ile Asp Val Val Val Gly Val Asp Ser Thr
230 235 240

Ala Gly Asn Asp Gly Thr Pro Asp Gly Leu Asp Thr Ala Ala His Arg
245 250 255 260

Trp Tyr Phe Gly Pro Thr Thr Glu Cys Ser Ser Ser
265 270

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<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligo 19671

<400> 11

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24

<210> 12

<211> 77

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligo 991222J1

<220>

<221> misc_feature

<222> (50)..(57)

<223> n is C or G or T or A

<400> 12
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acatgtcccc attaacc 77

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
2880 Bagsværd
DENMARK

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: NN049560	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: DSM 14047
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I. above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input checked="" type="checkbox"/> a proposed taxonomic designation</p> <p>(Mark with a cross where applicable).</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depository Authority accepts the microorganism identified under I. above, which was received by it on 2001-02-08 (Date of the original deposit).</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I. above was received by this International Depository Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s): <i>U. Weis</i> Date: 2001-02-19

Where Rule 6.4 (d) applies, such date is the date on which the status of international depository authority was acquired.

Form DSMZ-BP/4 (sole page) 0196

NZAS-0022451

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

PCT/DK02/00084

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
2880 Bagsværd
DENMARK

VIABILITY STATEMENT

issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Novozymes A/S Krogshøjvej 36 Address: 2880 Bagsværd DENMARK	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: DSM 14047 Date of the deposit or the transfer: 2001-02-08
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 2001-02-08. On that date, the said microorganism was <input checked="" type="checkbox"/> ¹ viable <input type="checkbox"/> ² no longer viable	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED ³	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s): <i>V. Wihs</i> Date: 2001-02-19

¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

⁴ Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
2880 Bagsværd
DENMARK

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: NN049561	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: DSM 14048
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I. above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input checked="" type="checkbox"/> a proposed taxonomic designation</p> <p>(Mark with a cross where applicable).</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depository Authority accepts the microorganism identified under I. above, which was received by it on 2001-02-08 (Date of the original deposit)¹.</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I. above was received by this International Depository Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s): <i>V. Wels</i>
Address: Mascheroder Weg 1b D-38124 Braunschweig	Date: 2001-02-19

¹ Where Rule 6.4 (d) applies, such date is the date on which the status of international depository authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

PCT/DK02/00084

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
2880 Bagsvaerd
DENMARK

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Novozymes A/S Krogshøjvej 36 Address: 2880 Bagsvaerd DENMARK	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: DSM 14048 Date of the deposit or the transfer: 2001-02-08
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 2001-02-08. On that date, the said microorganism was <input checked="" type="checkbox"/> viable <input type="checkbox"/> no longer viable	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED*	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s): <i>V. Warts</i> Date: 2001-02-19

- * Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
- * In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
- * Mark with a cross the applicable box.
- * Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
2880 Bagsværd
DENMARK

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: NN049562	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: DSM 14049
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I. above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input checked="" type="checkbox"/> a proposed taxonomic designation</p> <p>(Mark with a cross where applicable).</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depository Authority accepts the microorganism identified under I. above, which was received by it on 2001-02-08 (Date of the original deposit)¹.</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I. above was received by this International Depository Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s):  Date: 2001-02-19

¹ Where Rule 6.4 (d) applies, such date is the date on which the status of international depository authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
2880 Bagsværd
DENMARK

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Novozymes A/S Krogshøjvej 36 Address: 2880 Bagsværd DENMARK	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: DSM 14049 Date of the deposit or the transfer: 2001-02-08
III. VIABILITY STATEMENT	
<p>The viability of the microorganism identified under II above was tested on 2001-02-08. On that date, the said microorganism was</p> <p><input checked="" type="checkbox"/> viable <input type="checkbox"/> no longer viable</p>	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED ⁴	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s):  Date: 2001-02-19

¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

⁴ Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
2880 Bagsværd
DENMARK

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: NN049564	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: DSM 14051
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I. above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input checked="" type="checkbox"/> a proposed taxonomic designation</p> <p>(Mark with a cross where applicable).</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depository Authority accepts the microorganism identified under I. above, which was received by it on 2001-02-08 (Date of the original deposit)¹.</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I. above was received by this International Depository Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s): <i>V. Wels</i> Date: 2001-02-19

¹ Where Rule 6.4 (d) applies, such date is the date on which the status of international depository authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
2880 Bagsværd
DENMARK

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM
Name: Novozymes A/S Krogshøjvej 36 Address: 2880 Bagsværd DENMARK		Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: DSM 14051 Date of the deposit or the transfer: 2001-02-08
III. VIABILITY STATEMENT		
The viability of the microorganism identified under II above was tested on 2001-02-08. On that date, the said microorganism was <input checked="" type="checkbox"/> viable <input type="checkbox"/> no longer viable		
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED¹		
V. INTERNATIONAL DEPOSITORY AUTHORITY		
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig		Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s):  Date: 2001-02-19

¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

⁴ Fill in if the information has been requested and if the results of the test were negative.